Original Article

Microbial and Composition Changes during Vermicomposting Process Resulting from Decomposable Domestic Waste, Cow Manure and Dewatered Sludge

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Abstract

Aim: Although vermicomposting is rich in nutrients, the virulent microbes and pathogens present in it may be a threat to human health and the environment. Therefore, the objective of this study is to investigate the microbial quality of produced vermicompost, including fecal coliform and parasitic eggs, at a pilot scale, and compare it to present standards. **Materials and Methods:** Three various reactors containing decomposable domestic waste (T1), cow manure (T2), and dewatered sludge (T3) were used to produce vermicompost using *Eisenia fetida*. According to the standard methods, fecal coliforms, parasitic eggs, and some of the treatment characteristics including organic carbons, nitrogen, temperature, humidity, pH, electrical conductivity and metals were evaluated during the 56-day operation period. **Results:** According to the results, the number of fecal coliforms in treatments of T1, T2 and T3 reduced from 2.5×10^4, 6×10^5 and 15×10^6 to 1000, 1500 and 1500 MPN/g dw, respectively. All parasite eggs reached zero after the 3rd week. At the end of the study, the average of organic carbon in T1, T2, and T3 were 35.4 ± 6%, 50.7 ± 5%, and 58.4 ± 7%, respectively. This value for total nitrogen were 0.9 ± 0.2%, 1.8 ± 0.7%, and 4.2 ± 1.2%, respectively. **Conclusion:** Results showed that the worm *E. fetida* has a great ability to reduce pathogens without the need for an increase in temperature. Furthermore, it can be concluded that vermicompost can improve the quality of compost in 8 weeks. The vermicomposting process can also greatly destroy the fecal coliforms and all parasite eggs.

Keywords: Composting, *Eisenia fetida*, manure, parasites, solid waste

Introduction

Optimal management of urban and industrial waste, with regard to the large volume of its daily production in all countries, is of particular importance. Considering the limitation of proper sites for disposal of various waste, and the adverse effects of waste disposal on public health and the environment, progressing toward optimal management with an approach to sustainable development are the main objectives of developed and developing countries. The increasing production of waste has led to the invention of new and environmentally compatible methods for converting waste to beneficial products. Among these methods is the composting process, and the production of vermicompost.[1,2]

As a green technology, vermicomposting is a biochemical method which involves using worms for the conversion of waste to stable and high-quality organic material.[3] This process plays an essential role in the optimal management of waste, and in addition to decreasing economic, health, and environmental issues, it plays a very important part in producing organic matter and replacing hazardous chemical fertilizers, as well as ridding aquatic and terrestrial ecosystems from contamination caused by waste production.[4,5] In vermicompost, both earthworms and micro-organisms have an important role in the decomposition and stabilization of waste.[3] In comparison to regular compost, vermicompost

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has higher quality and can improve agricultural soil quality. Vermicompost is rich in nitrogen, phosphorus, sodium, micronutrients, and efficient microbes in the soil.[6] Vermicomposting is a semi-aerobic process which is performed by a specific group of worms and with the help of terrestrial bacteria and actinomycetes. According to studies, the most appropriate worm for this process is the earthworm, *Eisenia fetida*. This worm is a brown-reddish color and smaller than regular earthworms.[7,8] Due to the worms' bullet-shaped feces, vermicompost has less density than regular compost; this leads to increase in porosity, permeability, and ventilation of the soil. Furthermore, vermicompost stores more water due to its high field capacity, which prevents severe water stress in plants.[9,10]

So far, some studies have been conducted on the trend of pathogen elimination through the vermicomposting process. Monroy *et al*. (2009) reported an 85% reduction in total coliforms during the vermicomposting process of pig fertilizer.[11] Zhang *et al*. investigated the effect of temperature (10°C, 15°C and 25°C) on the growth of *E. fetida* during the vermicomposting procedure. They found that the worms' growth increases with the increase in temperature.[3,12] Hill and Baldwin conducted a study titled "Vermicomposting toilets, an alternative to latrine style microbial composting toilets," with the objective of recycling feces in a safe manner and producing humus. Their results indicated that the *Escherichia coli* count showed a 5 log reduction, whereas the number of parasitic eggs did not decrease.[13]

Although some studies have been conducted regarding the reduction of pathogens through the vermicomposting process, due to the widespread use of this method at a domestic and industrial level,[4,13-17] ambiguities still exist regarding the process of pathogen reduction. Therefore, the aim of this research is to investigate weekly trends of pathogens in vermicompost produced from domestic waste, sewage sludge, and cow manure.

Materials and Methods

Preparation of the treatments

This is a semi-experimental laboratory research, carried out at a pilot scale. Studied treatments include: (1) A reactor containing domestic decomposable waste such as food residues, vegetable and fruit waste (T1), (2) A reactor containing cow manure (T2), and (3) A reactor containing dewatered sludge (T3). Each treatment was carried out in two repetitions. *E. fetida* worms were used in the production of vermicompost. Considering the treatment surface and according to previous experience, 1000 worms (5–7 cm size) were added to each treatment.[18,19]

Each treatment was tested in a 15 cm × 30 cm × 50 cm porous plexiglass pilot (for better aeration of treatments) in shading conditions for 8 weeks. About 80% of the volume of each treatment container was covered with waste. This experiment was conducted in aerobic conditions and ambient temperature (30°C ± 5°C). Mechanical aeration was performed once daily in reactors. Water was added to the treatments twice daily to provide optimum moisture (60%–80%). To evaluate all parameters, three samples from each treatment were collected in each sampling. Some physico-chemical properties of waste material including temperature, moisture, pH, and electrical conductivity (EC) were evaluated daily. Evaluation of parasitic eggs and coliforms was also performed weekly. Total organic carbon and total Kjeldahl nitrogen were evaluated on days 1, 30, and 56.

Physic-chemical analysis

A pH/moisture meter (model PH MOISTURE) was used to measure PH and moisture; the temperature was measured using a soil thermometer (TFA model); EC was measured using a Conductivity Meter (Greisinger-Electronic model). Total nitrogen and organic carbon were also evaluated via the Kjeldahl method and 960A United States Environmental Protection Agency (EPA) method, respectively.[6,20]

Microbial analysis

Samples were collected in plastic bags for the microbial testing procedure. For determining the probable number of fecal coliform bacteria and parasitic eggs, A_1 and zinc sulfate specific culture media were used, respectively, according to the standard method.[21] After preparation of the mentioned culture media, 5 g of the sample was inoculated in peptone water – A liquid culture medium for nonselective enrichment – And after placing in an incubator shaker at 200 RPM (revolutions per minute) and 25°C for 5 min, it was moved to a 37°C incubator for 16–20 h. Coliforms were identified via the 9-tube fermentation technique and the A_1 medium. For the coliform test, which was also carried out via a multiple-tube fermentation technique, three dilutions of 1, 0.1, and 001 were used under serology bain-marie conditions at 41.5°C for 20–24 h. For identification of parasitic eggs, at first half, a tube was filled with zinc sulfate. Then, after transferring 1 g of the sample to a test tube, the suspension was prepared. The mentioned suspension was filtrated through double-layer gauze, and the filtered solution was returned to the tube. Zinc sulfate was added to the tube until the surface of the solution reached 2–3 mm from the tube opening. After centrifuging at a speed of 3000 rpm for 1 min, a loop from the surface of the tube's solution was removed and added to a microscopic slide containing Lugol's solution for microscopic identification.[19]

Statistical analysis

Data were analyzed using a Statistical Package for the Social Sciences (SPSS: An IBM Company, United States, New York). One-way analysis of variance was used for determining the impact of treatment on coliform count. The level of significance was considered 0.05.

Results

The average of some of the most important physicochemical characteristics of the treatments including temperature, humidity, pH, and EC are shown in Table 1. The trend of changes in the physicochemical properties of these treatments during the study is also shown in Figures 1-4. These characteristics include the percentage of humidity, temperature, pH, and EC.

The trend of changes in carbon and nitrogen levels in various treatments during the study is shown in Table 2.

According to results obtained from this study, there was a dramatic decrease in the number of fecal coliforms in all of the treatments, such that fecal coliform count in sludge, cow manure, and domestic waste treatments decreased from 15 × 10^6 MPN/g–1500 MPN/g, 6 × 10^5 MPN/g–1500 MPN/g, and 2.5 × 10^4 MPN/g–1000 MPN/g, respectively. All these differences were statistically significant ($P < 0.05$). According to results, raw sewage sludge samples contained the highest number of parasitic eggs (35/g). Through the vermicomposting process, this content was completely eliminated in the 3rd week. Fecal coliform and parasitic eggs in different treatments are shown in Tables 3 and 4.

At the end of the study, the amount of some components was also investigated. The results of this analysis are given in Table 5.

Discussion

Utilizing sewage treatment plant sludge and cow manure for improvement of soil quality carries the risk of transmitting pathogens to individuals; therefore, elimination of these agents is of great importance. Several studies have shown that using the vermicomposting process for the elimination of pathogenic agents and improvement of soil structure has been very successful.[4,13,18,19,22]

The effect of earthworms on the physicochemical properties of the treatments

Changes in parameters such as humidity, temperature, pH, and EC during the vermicomposting process are presented in Figures 1-4. Optimal humidity, for the accomplishment of biological functions and decomposition of organic materials via earthworms, is among the crucial factors. During the process, vermicompost humidity content must remain in the 75%–90% range.[4] As depicted in Figure 1, the humidity trend in all three reactors followed the same pattern and remained in the 60%–75% range.

In contrast to the composting process, vermicomposting is a mesophilic process which is implemented using micro-organisms and earthworms in a 10°C–32.2°C temperature range.[5,19] As illustrated in Figure 2, the temperature trend in all three treatments was almost identical, such that after initiation, the reaction showed an increasing trend, and maximum temperatures of 32°C, 33°C, and 35°C were obtained for domestic residues, cow manure, and sewage treatment plant sludge treatments, respectively, but temperature range at the end of the process was in 22°C–27°C.

pH trend during the vermicomposting process in the present study was investigated [Figure 3]. pH showed an increasing trend during the process, such that pH in the final product for treatments of T1, T2, and T3 was 7.5, 7.3, and 7.6, respectively.

Table 1: Mean of physic-chemical of the waste in various treatments

Parameter	Mean±SD		
	Domestic waste (T1)	Cow manure (T2)	Dewatered sludge (T3)
Humidity (%)	70.5±6.1	65.8±7	69±7
Temperature (°C)	27±3	28.5±4	26.5±3.8
pH	7.77±0.33	7.3±0.22	7.4±0.17
EC (mmhose/cm)	0.82±0.3	0.69±0.23	1.2±0.47

SD: Standard deviation, EC: Electrical conductivity

Table 2: Average of physico-chemical parameters of different treatments during the study

Parameter	Domestic waste			Cow manure			Dewatered sludge			Physic-chemical standard of compost, Iran (Grade 2)
	Day 1	Day 30	Day 65	Day 1	Day 30	Day 65	Day 1	Day 30	Day 65	
Organic carbon (%)	45.3±8	43.5±5	35.4±6	64.4±11.42	58.8±9	50.7±5	70.26±8	65±11	58.4±7	25 (minimum)
Nitrogen (%)	1.3±0.3	1.8±0.4	0.9±0.2	21.1±0.15	3.1±0.2	1.8±0.7	5.4±0.65	5.6±0.4	4.2±1.2	1-1.5

Table 3: Comparison of fecal coliform during the vermicompost process

Treatments	Raw sample	Week 1 (MPN/g)	Week 2 (MPN/g)	Week 3 (MPN/g)	Week 4 (MPN/g)	Week 5 (MPN/g)	Week 6 (MPN/g)	Week 7 (MPN/g)	Week 8 (MPN/g)
T1	2.5×10^4	1800	640	230	300	4600	11,000	11,000	1000
T2	6×10^5	4600	630	280	150	1500	11,000	11,000	1500
T3	15×10^6	11,000	2900	1600	300	2900	11,000	11,000	1500

Table 4: Comparison of parasitic eggs during the vermicompost process

Treatments	Raw sample (*n*/g)	Week 1 (*n*/g)	Week 2 (*n*/g)	Week 3 (*n*/g)	Week 4 (*n*/g)	Week 5 (*n*/g)	Week 6 (*n*/g)	Week 7 (*n*/g)	Week 8 (*n*/g)
T1	3	1	ND	ND	ND	ND	ND	ND	ND
T2	5	1	ND	ND	ND	ND	ND	ND	ND
T3	35	5	1	ND	ND	ND	ND	ND	ND

Table 5: Qualitative characteristics of vermicompost on day 56

Parameter	Mean±SD			Acceptable limit for compost (Iranian standard)
	Dewatered sludge	Cow manure	Domestic waste	
K (%)	1.3±0.4	1.1±0.5	1.5±0.6	NS
Na (%)	0.43±0.2	0.23±0.1	0.4±0.15	NS
Cu (%)	125±18.2	110±12.5	98±10	650 ppm
Zn (%)	720±102	420±87	560±55	1300 ppm
Fe (%)	1.1±0.45	0.97±0.35	1±0.3	NS
Mn	420±23	255±31	265±38	NS

NS: Not specified, K: Potassium, Na: Sodium, Zn: Zinc, Fe: Iron, Cu: Copper, Mn: Manganese, SD: Standard deviation

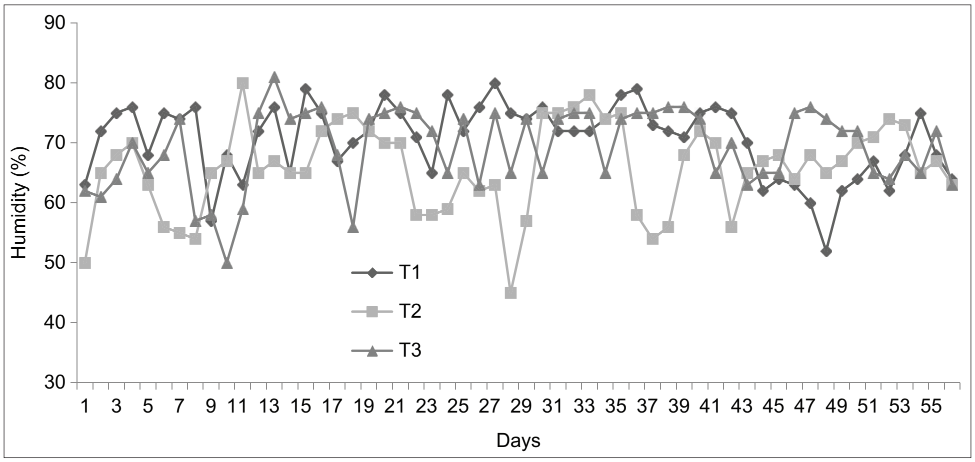

Figure 1: Moisture changes in various treatments during the study

Decomposition of organic materials by earthworms and microbes in the 1st week resulted in the production of ammonium and an increase in pH. However, at the end of the process, pH showed a slight decrease which is probably due to the metabolic activities of earthworms and the production of CO_2. The results of several studies are consistent with the results of the present study.[17,23]

EC trend is shown in Figure 4. According to results, EC showed an increasing trend in the first 4 weeks, but after that and until the end of the process, had a decreasing trend. EC in the final product of all three treatments was <0.5 mmho/cm. Increase in EC in the 1st week is probably due to the decomposition of organic material and the mineralization process which increases the number of ions and leads to an increase in EC, but over time, due to leakage of leachate containing mineral ions, EC decreases.[5,24] Huang *et al*. reported that in the first 2 weeks of the vermicomposting process, EC increased and then decreased, which is consistent with the results of the present study.[17] Also, Amouei *et al*. reported the reduction of EC in the final product.[23]

In the present study, organic carbon and nitrogen trends were measured on days 1, 30, and 56 [Table 2]. Results indicate that over time and during the process, organic carbon value decreases. Mineralization and decomposition by earthworms reduced organic carbon concentration in all three treatments.[16,22] Villar *et al*. reported the dramatic reduction of organic carbon value in the vermicomposting process.[25] Amouei *et al*. also reported similar results.[23]

The nitrogen concentration trend during the vermicomposting process is presented in Table 2. According to results, after reaction initiation, nitrogen value increased and then showed a decreasing trend, which is in line with the results of Huang *et al*.[17] Earthworms can excrete nitrogen through their skin and bodily excrements, which is probably one of the reasons for nitrogen increase in the initial days of vermicomposting. Furthermore, the death of some worms in the reactors, which were not capable of adapting to new treatments, may be another reason for nitrogen increase in the studied treatments. A reason for nitrogen decrease in the final stages of vermicomposting may be the decomposition of organic materials and the escape of nitrogen compounds along with leachate from the reactor. Leachate produced in the vermicomposting process has been mentioned as a rich source of nitrogen which could be a suitable source for improving soil property.[17,26]

The effect of earthworms on the microbial characteristics of treatments

The vermicomposting process was studied, with the aim of eliminating pathogens using different earthworm species and the endosymbiotic microbe population.[27] Coliform elimination rate during the vermicomposting process in all three treatments is reported in Table 3. According to results, it was evident that at the beginning of the process, fecal coliform had a

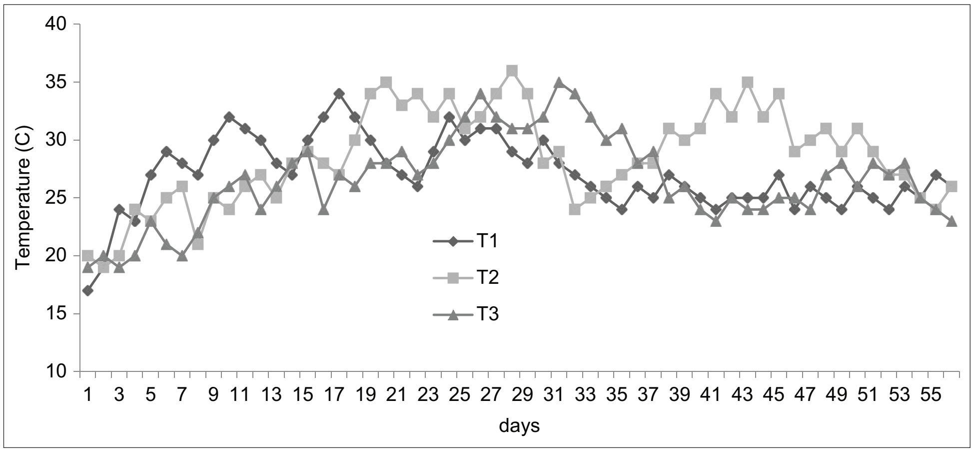

Figure 2: Temperature changes in various treatments during the study

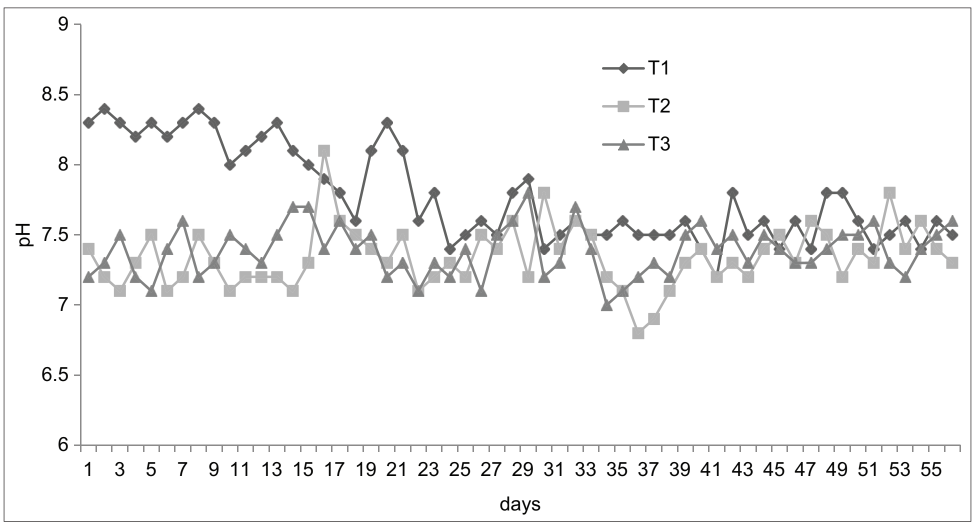

Figure 3: pH changes in various treatments during the study

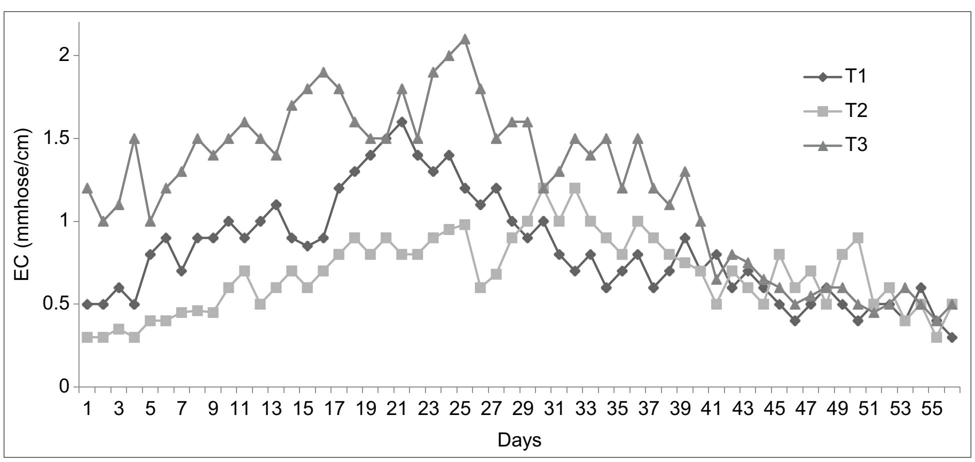

Figure 4: Electrical conductivity changes in various treatments during the study

decreasing trend, such that at week four it reached the lowest amount (300, 150, and 300 MPN/g for treatments of T1, T2, and T3, respectively), and after that, an increasing trend was observed where eventually, fecal coliform concentration in the final product was 1000, 1500, and 1500 in treatments T1, T2, and T3, respectively.

Parasitic egg elimination rate was also investigated in this study. Dewatered sludge (T3), with 35 parasitic eggs, had the highest count among the treatments. Results showed that 3 weeks after reaction initiation, the number of parasitic eggs reached zero in all three treatments. Different treatments passing through earthworm intestine affects the microbial population of treatments. The reduction of pathogens via the vermicomposting process depends on various factors such as intestinal enzyme activity in earthworms, coelomic fluid secretion which possesses anti-bacterial properties, as well as the competition between different groups of micro-organisms.[5,18] Studies have also shown that earthworms use nematodes as a food source and proteolytic enzyme activities eliminate nematodes and pathogens.[5,28]

Numerous studies corroborate our findings. Monroy *et al*. conducted a study to evaluate the effect of vermicomposting on pathogens and their results showed that pathogens had decreased drastically.[29] Furthermore, in this study, the number of coliforms increased after the 4th week which may be due to the lack of proper nutrients for earthworms, as well as humidity.[11] Rodríguez-Canché *et al*. utilized the vermicomposting process in a study with the aim of eliminating pathogens in septic tank sludge, and results showed that pathogen count had reduced drastically.[30] In a study conducted by Aira *et al*. on the reduction of pathogens in cow manure, results showed that fecal coliform had not reduced to the standard EPA level which is consistent with the results of the present study.[31] Edwards *et al*. reported that using the vermicomposting process, the number of fecal coliforms reduced drastically after 7 days. Their results also showed that 3 days after the vermicomposting process, the number of parasitic worms decreased significantly, which is in line with the results of the present study.[5] The results of Karimi *et al*. and Hait and Tare are also consistent with the results of the present study.[18,19]

Conclusion

This study aimed to investigate the effect of the vermicomposting process on the reduction of pathogenic load in sludge, cow manure, and domestic residue treatments. Results indicated that the vermicomposting process is capable of completely eliminating parasitic eggs in all three treatments. In conclusion, the vermicomposting process can be considered a suitable procedure for waste management, while the resultant products can be used for improving the properties of agricultural soil. It is recommended that other bacteria be tested under different operating conditions in future research.

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Conflicts of interest

There are no conflicts of interest.

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